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Studies on nicotine

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STUDIES ON NICOTINE

By

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PART I.

CHEMICAL TESTING OF NICOTINE DUSTS

INTRODUCTION

A very important advance in the use of nicotine preparations as an insecticide was the commercial introduction of nicotine dusts in about 1917. They consist of a very finely ground inorganic carrier such as kaolin or calcium carbonate with which is incorporated nicotine or nicotine sulfate. The use of these dusts has become quite extended in a comparatively short period of time.

Among the carriers used are hydrated lime, gypsum, calcium carbonate, dolomite, sulfur and various diatomaceous earths. These were found to vary in effectiveness and it soon became evident that there were a number of important factors which contributed to the insecticidal value of the dust. Among the more important are the rate of evolution of nicotine from the dust and its decomposition in the dust during storage.

De Ong (1) as a result of his work on volatility and toxicity of nicotine in sprays and dusts has concluded "that the volatilization curve of nicotine is almost an exact parallel of the curve of toxicity both of fumigation and spraying. Dust carriers follow the same law." Thus the dust which vola-

(1) de Ong, J. Econ. Entomol. 16:486 (1923).

tilizes the largest amount of nicotine within a specified time is the most effective as an insecticide.

Smith (2) appears to be the first investigator to test the efficiency of various carriers. He concluded that the toxicity of the dust is influenced by the nature and amount of nicotine present and by the diluent or carrier.

The most extensive work on the volatilization of nicotine from dusts is due to Thatcher and Streeter (3). They divide the carriers for nicotine sulfate into three groups: "Adsorbent substances" such as talc and kieselguhr which tend to prevent volatilization, "inert substances" such as calcium sulfate and sulfur which have no effect other than to expose large surfaces for evaporation, and "active substances" such as calcium hydroxide and carbonate which increase the volatility of nicotine by changing the sulfate into the free base. They divide the carriers for nicotine into two groups, "inert" and "adsorbent", the former being the most desirable for the preparation of active dusts.

Headlee and Rudolphs (4) and Rudolphs (5) have also tested the volatility of nicotine from several carriers and find indications of adsorption by the carriers which are col-

(2) Smith, Calif. Agr. Exp. Sta. Bull. 366 (1921).
(3) Thatcher and Streeter, N. Y. Agr. Exp. Bull. (Geneva) 501 (1923).
(4) Headlee and Rudolphs, J. Econ. Entomol. 15, 75 (1922).
(5) Rudolphs, J. Econ. Entomol. 15, 421 (1922).

loidal rather than crystalloidal in character. They state that for carriers impregnated with nicotine sulfate the evolution of nicotine is mainly dependent upon chemical reaction (decomposition of nicotine sulfate) but that physical factors only determine the evolution of the free base from carriers.

The volatility of nicotine from a dust must necessarily be limited by the vapor pressure of pure nicotine. The rate of attainment of equilibrium between the vapor and liquid nicotine or the carrier will also be a factor in volatilization. In commercial practice, dusting is carried out when there is as little air movement as possible. Under these conditions, the vapor concentrations in the vicinity of the dust should closely approach the equilibrium values of the dust. No quantitative data are available upon vapor concentrations obtained from pure nicotine or from dusts impregnated with nicotine.

McDonnell and Young (6) have determined the loss of nicotine from various dusts during storage. They report a wide variation in loss depending upon the type of container used. The work of Thatcher and Streeter (3) partially substantiates these results. De Ong (1) has reported decomposition of nicotine in the vapor phase. The failure to establish equilibrium

(6) McDonnell and Young, U. S. Dept. Agr. Bull. 1312 (1925).

under conditions similar to those used in determining vapor pressure by the dynamic method lead Hixon and Drake (7) to suppose that decomposition was occurring in dusts.

The purpose of the following experimental work was to determine the vapor concentrations over pure nicotine and over representative dusts and to investigate the nature of the decomposition and adsorption of nicotine by various carriers. The concentrations of nicotine vapor in equilibrium with pure nicotine have been determined by the air-bubbling dynamic method at 25°, 30°, 35°, and 40°C., this range including temperatures usually met in fumigation. The vapor concentrations of nicotine over a nicotine-hydrated lime dust and a nicotine-bentonite dust have also been determined.

(7) Hixon and Drake, Iowa State College J. of Sci. 1, 373 (1927).

EXPERIMENTAL

The apparatus consisted of a Mohr-Geissler potash bulb, a large test-tube tightly packed with glass-wool, and an absorption train consisting of two bubblers. Rubber connections were intentionally avoided to prevent adsorption of nicotine by the rubber.

Measured quantities of dry air were saturated with nicotine vapor by bubbling the air through pure nicotine contained in the Mohr-Geissler potash bulb and a 20 cm. test-tube tightly packed with glass-wool thoroughly moistened with nicotine. It was necessary to pass the air saturated with nicotine through glass-wool to eliminate the spray which passed over from the Mohr-Geissler bulb.

The nicotine vapor was adsorbed by washing the gases with a series of bubblers containing 2N sulfuric acid. Analysis was made by precipitating the solution with silico-tungstic acid, igniting and weighing the residue according to the A. O. A. C. (8) methods.

The volumes of air passed through the nicotine were measured with a calibrated flow meter. The rate was varied from 5 to 10 liters per hour, constant results being obtained showing that equilibrium had been attained. Temperature con-

(8) A. O. A. C., "Methods of Analysis" (Association of Official Agricultural Chemists, 1925) 67.

trol was maintained by an air thermostat to ± 0.5°C.

From the weight of nicotine obtained in the absorption train and the volume of air passed through, the concentrations of nicotine vapor were calculated in milligrams per ten liters of air and also in parts of nicotine per million of air assuming the ideal gas law for nicotine vapor. The barometric pressure was 740 mm. These data are reported in Table I.

Table I

Vapor Concentrations of Nicotine

Temperature °C	Air Passed over Nicotine (liters)	Nicotine Obtained (gram)	Concentration mg./10 liters of air	Concentration P.p.m.
25	60	0.01106	1.84	28.5
25	60	0.01056	1.76	27.3
30	60	0.01635	2.73	43.0
30	60	0.01596	2.66	41.9
35	30	0.01250	4.17	66.8
35	30	0.01232	4.11	65.9
40	30	0.01723	5.74	94.3
40	30	0.01812	6.04	99.2

The vapor pressure may be approximately calculated from the data given in Table I, but for practical purposes the concentrations as given in the table are more convenient for this type of work.

Volatility of Nicotine in Lime and Bentonite Dusts

The apparatus consisted of a humidity control, mixing bottle, and flow meters to regulate the volume of air passed over, being essentially the apparatus described by Hixon and Drake (7).

The dusts were prepared with hydrated lime and bentonite as carriers. These materials were sieved, that portion being used which passed a 100-mesh and was retained by a 200-mesh sieve. The size of particles of a carrier undoubtedly influences the rate of attainment of equilibrium of the vapor phase with the liquid phase on the dust particles but should not influence the value of this equilibrium unless there is not sufficient nicotine present to completely take care of the adsorption by the dust and leave unadsorbed liquid nitotine on the surface of the particles.

The bentonite was a commercial product having the following analysis:

SiO_2	60.64%
Al_2O_3	23.26
Fe_2O_3	3.92
TiO_2	0.12
CaO	0.59
MgO	2.19
K_2O	0.37
Na_2O	4.33
H_2O	2.83
Total . . .	98.25%

Pure nicotine was used for the preparation of the dusts,

analyses showing 2.97 per cent nicotine in the hydrated lime dust and 2.99 per cent nicotine in the bentonite dust.

Measured quantities of air were passed over a sufficient quantity (20 to 30 grams) of the dust to give equilibrium conditions. No consistent variation in results was obtained by passing air over the dust at different rates (5 to 10 liters per hour) showing that the nicotine vapor was in equilibrium with the liquid nicotine on the dust. The data on the nicotine-hydrated lime dusts is given in Table II.

Table II

Vapor Concentrations 2.97 Per Cent of Nicotine-hydrated Lime Dust at 35°C.

Number	Air Passed Over Dust (liters)	Nicotine Obtained (gram)	Concentration mg./10 liters air	Concentration P.p.m.
1	60	0.01971	3.28	52.5
2	30	0.00940	3.13	50.1
3	30	0.01025	3.42	54.8
4	30	0.00892	2.97	47.6

These measurements were made at different times over a period of three months. The values are considerably less than those for pure nicotine at the same temperature as a comparison of Tables I and II will show.

Similar measurements made on the 2.99 per cent nicotine-bentonite dust revealed a concentration of nicotine too small

to determine, only the slightest trace of nicotine vapor being detected on passing air over the dust. This is in accord with the strong adsorptive properties exhibited by bentonite.

Adsorption of Nicotine by Bentonite

Previous to preparing a dust, the bentonite was extracted with ether to remove any ether-soluble material which it might contain. The nicotine was a colorless oil having a boiling point of 247°C. at 730 mm. and a density of 1.010 at 20°C.

A dust containing 3 per cent nicotine was prepared and samples allowed to remain exposed to the air for several days (nicotine is comparatively non-volatile from bentonite, the odor of nicotine completely disappearing in three days in closed containers) while other samples were kept in tightly stoppered bottles.

The samples of nicotine-bentonite dust were then extracted with ether for twenty-four hours in a Soxhlet extractor. In both the dusts exposed to the air and those in stoppered containers only 10% to 20% of the nicotine originally present was obtained by extraction for forty-eight hours with ether. An examination of this ether extract failed to reveal any decomposition products of nicotine. The ether soluble oil reported by Hixon and Drake (7) was found to have

come from outside contamination during the extractions.

The ether-extracted dust was steam distilled and in all cases the remainder of the nicotine was quantitatively recovered. An examination of the steam distillate showed no decomposition products.

The amount of nicotine adsorbed or held by bentonite is approximately a constant amount regardless of the concentration of nicotine in the dust. In a 3% dust 12% of the nicotine is extracted by ether, in a 6% dust 54% is extracted and in a 14% dust 80% is extracted; that is, in a 3% dust 0.0264 gm. of nicotine is retained per gram of bentonite, in a 6% dust 0.0276 gm. is retained and in a 14% dust 0.0280 gm. is retained. These data were obtained by extracting the nicotine-bentonite dusts for twelve hours in a Soxhlet extractor. The addition of hydrated lime (10% to 20%) increases the amount of nicotine extracted by ether.

Very little nicotine is obtained in the filtrate when a 3% nicotine or nicotine sulfate-bentonite dust is washed with water. It is also noteworthy that considerable heat is evolved when pure nicotine is added to bentonite which is indicative of an adsorption or union between the bentonite and nicotine.

Bentonite and hydrated lime dusts have shown no significant loss of nicotine (less than 0.1%) when kept in tightly

stoppered bottles for six months. It should be pointed out, however, that the silico-tungstic acid method of analysis is a poor criterion for decomposition of nicotine. Any compound which is volatile with steam and similar in structure to nicotine such as metanicotine will be precipitated along with nicotine by silico-tungstic acid. Thus only radical changes in the nicotine molecule (which are improbable in dusts) or the formation of compounds non-volatile with steam will be noted by the present analytical methods.

Adsorption of Nicotine in Vapor State

It is stated by De Ong (1) that as nicotine vapor diffuses in the air it is soon oxidized. As evidence of this he cites an experiment in which air was aspirated through a nicotine solution and the nicotine vapor passed through two or three feet of rubber tubing. Only a trace of nicotine vapor was recovered in the washing solution at the end of the tube, while a close correlation was found between the amount of nicotine recovered at the top of the tube and the concentration of the original solution.

Chemically nicotine is comparatively stable towards weak oxidizing agents. It is a common characteristic of many nitrogenous bases with a pyridine nucleus to darken when exposed to light or air without any appreciable decomposition taking place. Nicotine is oxidized to oxynicotine by a 2-1/2%

solution of H_2O_2 (9)(10). This is the most logical oxidation product of nicotine with air acting as the oxidizing media. No trace of this compound has been found in dusts exposed to the air or in nicotine vapor-air mixtures.

Nicotine vapor from a nicotine-hydrated lime dust (using apparatus described by Hixon and Drake (7) was passed through a series of 3-2 liter glass bottles. These bottles were closed with rubber stoppers with an inlet and outlet glass tube, these tubes being connected together with rubber tubing. Air was passed over the dusts at six liters per hour. One hour would then elapse from the time the nicotine entered the series of bottles until it was absorbed for analysis by washing the gases in 2N H_2SO_4 with a series of bubblers. Time was allowed in all cases for any ordinary adsorption on the walls of the bottles to take place before any analyses were made. Analyses of the nicotine concentration were made before and after the vapor had passed through this series of bottles. A large decrease in concentration was always noted. This difference in nicotine concentration was also noted biologically. Rice weevil (Calendra oryzae L.) were placed in each of the bottles in the series. Much larger kills were

(9) Pinner and Wolffenstein, Ber. 24, 61 (1891).
(10) Auerbach and Wolffenstein, Ber. 34, 2411 (1901).

always obtained in the bottles nearest the source of nicotine vapor.

These observations are in harmony with de Ong's conclusions mentioned above but when an entire glass train with no rubber stoppers or connections was inserted no such discrepancies were found. In Table III data are given upon which these statements are based. In numbers 1 and 2 the nicotine vapor was passed through a train containing rubber connections while in numbers 3, 4, and 5 the vapor was passed through an entire glass train. One hour was required for the nicotine vapor to pass through the train in experiments number 1 and 2 while only forty minutes were required in numbers 3, 4, and 5.

Table III

Experiment Number	Conc. Nicotine at beginning of train mg./10 L. air	Conc. Nicotine at end of train mg./10 L. air	% Nicotine not accounted for
1	2.56	1.52	-50.6%
2	1.56	0.90	-42.3%
3	2.67	2.66	- 0.4%
4	2.99	2.97	- 0.6%
5	2.76	2.78	+ 0.7%

These experiments were duplicated using pure nicotine as the source of nicotine vapor in place of the dusts with simi-

lar results. These data show that there is no rapid oxidation of nicotine vapor as suggested by de Ong, his conclusions being due to the adsorption of nicotine by rubber.

DISCUSSION OF RESULTS

Evidence has been presented which shows that bentonite adsorbs nicotine so firmly that it cannot be quantitatively extracted from the dust with either ether or water. It can, however, be quantitatively recovered by direct steam distillation of the dust. The fact that the vapor pressure of nicotine over a bentonite dust is so slight, the fact that there is no perceptible odor of nicotine in a 3% nicotine-bentonite dust two or three days after preparation when kept in tightly stoppered bottles, and the fact of the relatively large heat of adsorption when pure nicotine is added to bentonite, together with the insolubility of nicotine in ether and water indicate that the degree of adsorption is so great that it approaches a loose chemical union.

It would appear that much of the evidence presented for decomposition of nicotine in dusts and in the vapor state might be due to adsorption by different materials. Any experimental method which permits the contact of nicotine vapor with rubber (i.e. the storage of nicotine dusts in rubber stoppered bottles) or which reports the analysis of a dust by extraction with either water or ether without quantitative recovery cannot be interpreted as due to decomposition without further evidence.

SUMMARY

1. The vapor concentration of nicotine over the pure liquid increases from 1.76 mg. per 10 liters of air at 25°C. to 6.04 mg. at 40°C.

2. The vapor concentration of nicotine over a 2.97 per cent nicotine-hydrated lime dust lies between the limits of 2.97 to 3.42 mg. per 10 liters of air at 35°C. The corresponding value for the pure liquid is 4.14 mg. per 10 liters.

3. The vapor concentration of nicotine over a 2.44 per cent nicotine-bentonite dust was too small to measure, only slight amounts of nicotine being detected in the vapor phase.

4. A chemical study has failed to reveal any decomposition of nicotine in dusts or in the vapor phase.

5. Carriers such as bentonite adsorb nicotine so strongly that it cannot be extracted by water or ether.

6. Rubber shows very high adsorptive powers toward nicotine and must be eliminated from all portions of the experimental apparatus.

PART II.

TOXICITY OF NICOTINE AND RELATED DERIVATIVES

INTRODUCTION

Insecticides are usually divided into two main groups: Stomach poisons which are introduced by way of the alimentary canal and contact poisons which are introduced through the respiratory tract or cuticle. This division is arbitrary as many insecticides are both stomach and contact poisons, e.g., nicotine. Jannisch (11), while admitting the practical utility of this classification, considers it misleading on a physiological basis, his view being that there is little difference in physiological action between stomach and contact poisons, death or narcosis being due to an alteration of the colloidal condition of the protoplasts of the cell.

Contact insecticides may be used either as sprays or fumigants. The latter has much application in greenhouses or any closed space. Certain classes of contact poisons even when sprayed are said to act chiefly in the vapor state. McIndoo (12) claims that nicotine when sprayed gains entrance in this way and that its toxicity is primarily due to the vapor. De Ong (1)(13) has shown that the toxicity and volatility

(11) Jannisch, Centr. f. Bakt. 2, Abt. 61, 10 (1924).
(12) McIndoo, J. Agr. Res. 7, 89 (1916).
(13) de Ong, Ind. Eng. Chem. 16, 1275 (1924).

curves of nicotine parallel one another very closely.

It is surprising considering the large number of both synthetic and natural compounds tested as contact poisons that so few have been found with really outstanding toxic action upon insects. It would appear that nicotine, the pyrethrins and rotenone (from derris) are in a class by themselves in this respect. These compounds are of a widely different nature, nicotine being an alkaloid, the pyrethrins are both complicated esters containing a number of unsaturated bonds and asymmetric carbon atoms. The constitution of rotenone has not been determined as yet.

It has generally been conceded that a substance having a high toxic effect upon the higher animals would act equally well as an insect poison. For this reason most insecticides, especially the stomach poisons, have almost always consisted of inorganic metallic compounds, particularly compounds of copper, lead and calcium combined with arsenic in some form. It is uncertain whether the toxic action of chemical individuals upon insect metabolism is similar to the results obtained in mammalian toxicology. Investigations have been published from time to time showing that a particular material may be highly toxic to insects and have a low order of toxic action on the higher animals or conversely be highly toxic to the higher animals and of low toxicity to insects. This, however, has

not widely recognized in insecticidal research.

Nicotine was first isolated from the leaves of the tobacco plant in 1828 by Posselt and Reimann (14) and the empirical formula was established as $C_{10}H_{14}N_2$ by Melsens in 1849 (15). The quantity found in tobacco varies from 0.6 per cent to 10 per cent.

Nicotine is a colorless oil which boils without decomposition at 245° but does not solidify at -30°C., its specific gravity being 1.010 at 20°C. In the pure condition it is almost odorless but acquires the tobacco-like odor after standing for sometime. The free alkaloid is strongly laevo-rotatory, $[\alpha]_D^{20} = 168.2°$, but its salts are dextro rotatory. Through heating the mono-hydrochloride or sulfate of nicotine at 180-250°C. for several hours, inactive nicotine results which possesses all of the chemical and physical properties of the naturally occurring base other than optical activity.

The constitution of the alkaloid was first established by Pinner (16) as β-pyridylα-N-methyl pyrrolidine, after many years of controversial research. His structure was confirmed by the synthesis of inactive nicotine by Pictet (17) in 1904.

Nicotine is one of the most deadly and rapid poisons known.

(14) Posselt u. Reimann, Magazin f. Pharmazie 24, 138 (1828).
(15) Melsens, Ann. 49, 353 (1849).
(16) Pinner, Ber. 26, 292 (1893).
(17) Pictet and Rotschy, Ber. 37, 1225 (1904).

Sollman (18) states that the vapor arising from a glass rod placed near the beak of a small bird causes it to drop dead at once, and two drops placed on the gums of a dog may cause a similar result. On account of its high toxicity to insects it has come to be used as the standard contact insecticide.

The physiological action of nicotine has been thoroughly investigated. Greenwood (19) found that the toxic effect of nicotine on any organism is determined largely by the degree of development of the nervous system, those in which the nervous system is only slightly developed being the least affected. Cushny (20) in summarizing its physiological action on man and other mammals asserts that it causes a hot burning sensation in the mouth, which is followed by salivation, nausea, vomiting and sometimes purging. Mental confusion, muscular weakness and giddiness are followed by loss of coordination and partial or complete unconsciousness. Death is caused by the paralysis of the respiratory center.

Shafer (21) ascertained that nicotine vapor caused insects to pass through a stage of excitement, then through a stage of depression in which movement is uncertain and finally through a stage in which there was total loss of movement and sensibility. The last stage was followed rather closely by death.

(18) Sollmann, A Manual of Pharmacology (W. B. Saunders Co. 1917) 316.
(19) Greenwood, J. Physiol. 11, 573 (1890).
(20) Cushny, Pharmacology and Therapeutics (Lea & Febriger 1918) 309.
(21) Shafer, Mich. Agr. Exp. Sta. Tech. Bull. 2 (1911).

McIndoo (12) concludes that regardless of how nicotine is applied to insects as well as other animals it kills by paralysis, preventing the nerve cells from functioning in their normal manner. The paralysis in insects travels along the ventral nerve cord from the abdomen to the brain.

A review of the physiological action of the constituent parts of the nicotine molecule is pertinent. Pyridine (22) has slight effects upon the sensory nerves, respiration, and heart action but it may be considered as being comparatively non-poisonous. Its activity is increased by the addition of aliphatic side chains, especially alkyl groups, and its effect upon the sensory nerves is particularly increased (23). In the series pyridine, picoline (α-methyl pyridine), lutidine (α, α'-dimethyl pyridine), and collidine (2,4,6 trimethyl pyridine) there is an increase in activity which is manifest by an intoxicant effect.

The influence of hydrogenization upon physiological action is demonstrated in a marked degree in the cyclic bases. As we pass from pyridine, a comparatively non-poisonous base to piperidine (hexahydro pyridine) we find a marked increase in toxicity (24). It acts as a cramp-producing poison which

(22) Brunton and Tunicliffe, J. Physiol. 17, 272
(23) Kendrick and Dewar, Proc. Roy. Soc. 22, 432 (1873).
(24) Spiegel, Chemical Constitution and Physiological Action (D. Van Nostrand Co. 1915) 100.

considerably increases the blood pressure and finally causes paralysis of both central and peripheral nerves. The toxicity of piperidine is also markedly increased by the substitution of alkyl side chains. The toxic effect rises from piperidine to pipecoline (α-methyl piperidine), to α-ethyl piperidine, to coniine (α-propyl piperidine) in the ratio 1:2:4:8. Gamma substitution products increase in toxicity in a similar manner up to the propyl derivative, the isobutyl showing a decrease and the hexyl a still greater decrease.

The position in which the radical is substituted in the nucleus also influences the toxic action. The lethal dose of β -propyl piperidine is nearly twice as great as that of α-propyl piperidine (24), and the lethal dose of β-ethyl piperidine is twice as great as α-propyl piperidine (25)(26).

Alkylation on the nitrogen generally results in an enhanced physiological activity which is even greater than that caused by alkylation on a carbon atom. Here again there is a rise in toxicity up to the propyl radical and then a decrease in the higher homologues.

The derivatives of quinoline (24) in general have a less toxic effect than in the corresponding pyridine derivatives. In other words the condensation of a benzene ring with the

(25) Ehrlich and Granger, Ber. 30, 1060 (1897).
(26) Gunther, Ber. 31, 2141 (1898).

pyridine ring lessens its action. But toxicity increases from quinoline to tetrahydroquinoline to decahydroquinoline (27). The same generalization holds for isoquinoline and its hydrogenated bodies.

The influence of hydrogenation of cyclic bases is again demonstrated in the case of pyrrole. Pyrrole is itself a poison causing a central nervous paralysis (28) which is markedly increased on hydrogenation to pyrroline and is still further increased in pyrrolidine. Pyrrolidine acts qualitatively like piperidine and cyclohexamethyleneamine. Quantitatively cyclohexamethyleneamine is more toxic than piperidine which is in turn more toxic than pyrrolidine.

Summing up these generalizations for the physiological action of the simpler nitrogen heterocyclic compounds on the higher animals: toxicity is increased by increasing the size of the ring, by substitution of alkyl groups on both the carbon and nitrogen atoms and by hydrogenation.

From the limited data available on the toxicity of nitrogen heterocyclic compounds as contact insecticides the generalizations made for the higher animals appear to be substantiated with few exceptions. Neither pyrrole nor pyridine have

(27) Heinz, Virchow's Arch., 122, 116 (1890).
(28) Spiegel, Chemical Constitution and Physiological Action (D. Van Nostrand Co. 1915) 94.

any marked toxic action (29)(30), but hydrogenation of pyridine to piperidine and pyrrole to pyrrolidine increases their toxicity considerably, both being relatively highly toxic when compared to their parent substances. The substitution of alkyl side chains on the α, β, and γ carbon atoms increases toxicity markedly.

The choline bases of pyridine were more than twice as toxic as pyridine. Substitution of alkyl groups on the nitrogen atom of piperidine, however, did not materially alter its toxicity. Coniine (α-propyl piperidine) although quite poisonous to mammals is only slightly toxic to insects. Quinoline and isoquinoline are quite toxic to insects but only slightly so to the higher animals.

The toxicity of the simpler nitrogen heterocyclic compounds is in the following order (30): pyrrole < pyridine < picoline < lutidine < quinoline and isoquinoline < acridine.

From the data available the same generalizations appear to hold for the toxicity to insects as to the higher animals, namely, toxicity is increased by increasing the size of the ring, by substitution of alkyl groups on both carbon and nitrogen atoms (in some cases) and by hydrogenation, although Richardson and Smith (29) have shown that hydrogenation de-

(29) Richardson and Smith, U. S. D. A. Bull. 1160 (1923).
(30) Tattersfield and Gimingham, Ann. Appl. Biol. 14, 217 (1927).

creases toxicity in some cases.

Smith (31), and Richardson and Smith (32) have prepared and tested a number of the isomeric dipyridyls and find the crude mixture containing principally $\alpha\alpha'$, $\beta\beta'$, and $\beta\gamma$ dipyridyls more toxic than nicotine to some species of insects (larvae of Leptinotarsa decemlineata and Lema trilineata)but less so to Aphis rumicis. The and $\beta\beta'$ dipyridyls appeared to be more toxic than the $\beta\beta'$ dipyridyl and the $\gamma\gamma'$dipyridyl was much less toxic than the other three compounds. Tattersfield (30) has found the benzyl pyridines to have a high order of toxicity to Aphis rumicis.

LaForge (33)(34) in an effort to determine whether the pyrrolidyl or pyridyl portion of the nicotine molecule was responsible for its insecticidal activity has synthesized a number of substituted pyrrolidine and pyridine derivatives. None of these approach nicotine in toxicity and he concludes that from existing data it is impossible to arrange a list of compounds of progressively increasing toxicity up to nicotine.

The purpose of this investigation was to determine whether or not the toxicity of nicotine was due to the special configuration of the nicotine molecule or was an accumulation of the

(31) Smith, J. Am. Chem. Soc. 47, 414 (1924).
(32) Richardson and Smith, J. Agr. Res. 33, 592 (1926).
(33) LaForge, J. Am. Chem. Soc. 50, 2471 (1928).
(34) LaForge, J. Am. Chem. Soc. 50, 2477 (1928).

toxicities of methyl pyrrolidine and pyridine. From the toxicity of these constituent parts, the greater part of the toxicity should be grouped in the methyl pyrrolidine portion of the molecule rather than in the pyridine portion. The reduction products of nicotine have been prepared and their toxicities compared to that of nicotine. Several other degradation products of nicotine have also been prepared and their toxicities tested.

THE CATALYTIC REDUCTION OF NICOTINE

Introduction

Nicotine was first reduced by Liebrecht (35) with sodium and absolute alcohol. Since he regarded nicotine as a hexahydrodipyridyl he described the product of his reduction as $\beta\beta'$ dipiperdyl.

Blau (36) showed that the majority of the reduction product consisted of octohydronicotine (β -pyridyl N-methyl α-butyl amine) with a small amount of other nitrogenous bases, one of which he later identified (37) as hexahydronicotine (β -piperidyl α-N-methyl pyrrolidine). Pinner (38) although correctly establishing the structure of nicotine considered that the above reduction yielded hexahydronicotine entirely. For this reason there has been much confusion in the literature regarding the products obtained by the reduction of nicotine with sodium and alcohol.

Blau (37) separated hexahydronicotine in small quantities from the octohydrogenated base by steam distillation, the former compound being much more volatile with steam than the latter. It was further purified through fractional crystalliza-

(35) Liebrecht, Ber. 18, 2969 (1885).
(36) Blau, Ber. 26, 628 (1893).
(37) Blau, Ber. 26, 1029 (1893).
(38) Pinner, Ber. 26, 765 (1893).

tion of the chloroplatinates and finally of the picrates. This method of obtaining hexahydronicotine was too long and tedious and it proved practically impossible to prepare sufficient of the compound for toxicity tests.

Catalytic reduction appeared to offer a possible solution of the problem if the reduction could be carried out without rupturing the pyrrolidine ring as is the case in the reduction with sodium and absolute alcohol.

Hamilton and Adams (39) have reduced pyridine and quinoline hydrochloride in absolute alcohol with hydrogen and platinumoxide platinum black. They report unsatisfactory results with 95% alcohol as a solvent. However, 95% alcohol proved a very satisfactory solvent for the reduction of nicotine hydrochloride.

For the reduction platinum-oxide platinum black prepared according to the directions of Adams and Shriner (40) was used.

Nicotine hydrochloride was prepared from a commercial 95% nicotine solution. This was reduced with hydrogen and platinum-oxide platinum black to give a mixture of hexahydronicotine hydrochloride and octohydronicotine hydrochloride. No reduction was obtained in a variety of solvents with the free base, poisoning of the catalyst apparently taking place.

(39) Hamilton and Adams, J. Am. Chem. Soc. 50, 2260 (1928).
(40) Adams and Shriner, J. Am. Chem. Soc. 45, 2171 (1923).

Experimental

Nicotine hydrochloride was prepared by passing dry hydrochloride into a dry ethereal solution of commercial water-free 95 per cent nicotine. A small amount of acetone present served to aid crystallization of the nicotine hydrochloride. After precipitation was complete, the nicotine hydrochloride was rapidly filtered on a Buchner funnel and recrystallized from a mixture of absolute alcohol and acetone. The hydrochloride was taken up in a small amount of hot absolute alcohol and acetone added while the solution was still hot until it just became turbid. Seeding at this stage aids materially in obtaining crystals rather than an oil. After cooling the hydrochloride was rapidly filtered on a Buchner and again recrystallized according to the above procedure. The recrystallization of the nicotine hydrochloride by this method yields a very pure nicotine salt from a commercial product without numerous extractions and distillations. Because of the very deliquescent nature of nicotine hydrochloride it was stored in a desiccator over calcium chloride or stick sodium hydroxide, the latter serving to remove any occluded hydrogen chloride.

Reduction was carried out in an apparatus similar to that used by Adams and students in their reduction investigations (41).

(41) Adams and Voorhees, Org. Syntheses 8, 10 (1928).

As a typical run 0.1 mole of nicotine hydrochloride in 150 cc. of 95% ethyl alcohol with 0.2 gm. of the platinum-oxide platinum black from C.P. chloroplatinic acid was used. The catalyst was added to the reduction solution without previous reduction with hydrogen in all cases. The time required for complete reduction varied from three to four hours.

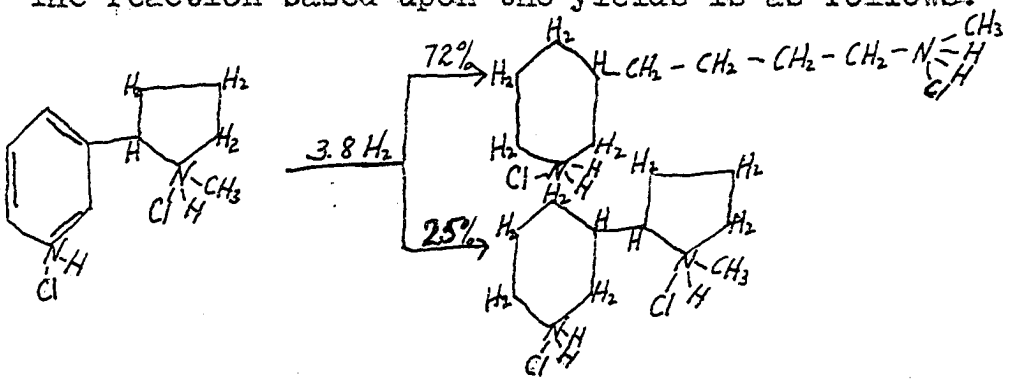
If the reduction were allowed to go to completion approximately four moles of hydrogen were taken up, indicating that the pyrrolidine ring was ruptured during the reduction. After removing the catalyst by filtration and evaporating the solvent under reduced pressure, a thick syrup was obtained which on taking up in absolute alcohol and acetone similar to the procedure followed for the purification of nicotine hydrochloride yielded a portion of the reduction products in crystalline form. This was filtered off and the filtrate evaporated leaving an oily residue. This procedure was repeated with a smaller yield of crystals. The oily portion which failed to crystallize is apparently more soluble in a cold alcohol-acetone mixture than in a hot one and consequently may be separated from the crystalline portion almost quantitatively. The crystalline product melted at 202°C. corresponding to the hydrochloride of octohydronicotine.

The picrate of the oil which failed to crystallize was prepared by adding a saturated 95% alcohol solution of picric

acid to an alcohol solution of the oil. An abundant yield of a crystalline picrate was obtained which was recrystallized from 95% alcohol. The picrate melted at 202°C. after repeated crystallization from both alcohol and water corresponding to the picrate of hexahydronicotine reported by Blau (37). The hydrochloride of hexahydronicotine is extremely hygroscopic and difficult to obtain crystalline.

Any octohydronicotine which was not removed before making the picrate may be recovered as the picrate in the combined filtrates from the crystallization of the hexahydronicotine picrate, the picrate of octohydronicotine being soluble in alcohol but insoluble in water, while hexahydronicotine picrate is practically insoluble in the cold in both alcohol and water.

The reaction based upon the yields is as follows:



Another reduction was carried out in which the reaction was stopped after 3 moles of hydrogen had been adsorbed to determine whether or not the rupture of the pyrrolidine ring was taking place before or after hydrogenation of the pyridine nucleus. The yields of the products isolated were: octohy-

dronicotine hydrochloride 57.1%, hexahydronicotine hydrochloride 19.0%, and unchanged nicotine hydrochloride 21.0%. Since the hexa- and octohydrogenated bases are formed in the same ratio as in the previous reaction, rupturing of the pyrrolidine ring is undoubtedly taking place simultaneously with hydrogenation.

In one of the runs where the reaction was stopped after 3 moles of hydrogen had been adsorbed a small amount of a picrate melting at 162°C. was obtained. This corresponded to the picrate of dihydrometanicotine and would seem to indicate that the rupturing of the pyrrolidine ring when it does take place is the first step in hydrogenation. This could not be checked in all runs, however.

The free bases may be obtained from the hydrochlorides by treating with sodium hydroxide and extracting with ether. After evaporating the ether, the free base may be distilled under atmospheric or reduced pressure. To obtain the free base from the picrate, the best procedure was found to be to treat the picrate with a small amount of 20 percent hydrochloric acid, which hydrolyzes the picrate almost immediately with the precipitation of a large part of the picric acid. This is filtered off and the remainder which stays in solution is removed by extracting the acid solution with ether. The solution is then made strongly basic and the free base extracted with

ether. The ether is removed by evaporation and the base distilled under atmospheric or reduced pressure.

In the identification of compounds, it was not possible to take the melting point of the hydrochloride in most cases due to their deliquescent nature. A double salt, usually with picric acid, was made and the melting point of this determined. The boiling points of the free bases were obtained in all cases with the exception of dihydrometanicotine.

The constants of these compounds are given in Table IV.

Table IV

Constants of and References to Nicotine and Reduced Products

Compound	HCl Salt M.P. °C.	Ref.	Base B.P. °C	Ref.	Derivative M.P. °C.	Ref.
$C_{10}H_{14}N_2.2HCl$	---		246	(b)	218 (picrate)	(d)
$C_{10}H_{20}N_2.2HCl$	---		246	(c)	202 (picrate)	(c)
$C_{10}H_{22}N_2.2HCl$	202	(a)	259	(a)	144 (dibenzene sulfonyl-chloride)	(c)

(a) Blau, Ber. 26, 628 (1893).
(b) Wolffenstein, Pflanzenalkaloide(Verlag von Julius Springer 3rd ed. 1922) 136.
(c) Blau, Ber. 26, 1029 (1893). The base obtained is a solid melting at 36-37°C.
(d) Pinner and Wolffenstein, Ber. 24, 66 (1891).

THE CATALYTIC REDUCTION OF METANICOTINE

Introduction

Metanicotine (β -pyridyl-N-methyl α -butyl amine) has been reduced with hydrogen iodide and phosphorus to dihydrometanicotine (42) and with sodium and absolute alcohol to a mixture of octohydrometanicotine and hexahydrometanicotine (43). The octohydrometanicotine is the same product as is obtained on the complete reduction of nicotine.

Metanicotine may be reduced catalytically with hydrogen and platinum-oxide platinum black with the production of octohydronicotine (octohydrometanicotine) or dihydrometanicotine depending upon the amount of hydrogen the reduction mixture is allowed to absorb.

The metanicotine for the reduction was prepared according to Loffler and Kober's modification (44) of Pinner's synthesis (45). This consists in heating nicotine in a large excess (1½ to 2 times the weight of nicotine used) of benzoyl chloride when benzoyl metanicotine is obtained. After purification, the benzoyl metanicotine is hydrolyzed with 20 per cent hydrochloric acid at 100°C. for twelve to twenty-four hours.

(42) Maass and Zablinski, Ber. 47, 1164 (1914).
(43) Maass and Hildebrandt, Ber. 39, 3697 (1906).
(44) Loffler and Kober, Ber. 42, 3431 (1909).
(45) Pinner, Ber. 27, 1053 (1894).

The separated benzoic acid is removed by filtration and the filtrate made strongly basic. The metanicotine separates and is extracted with ether. The ether is vaporized and the metanicotine distilled under atmospheric or reduced pressure. Prepared in this manner, metanicotine is a colorless oil boiling at 276°C.

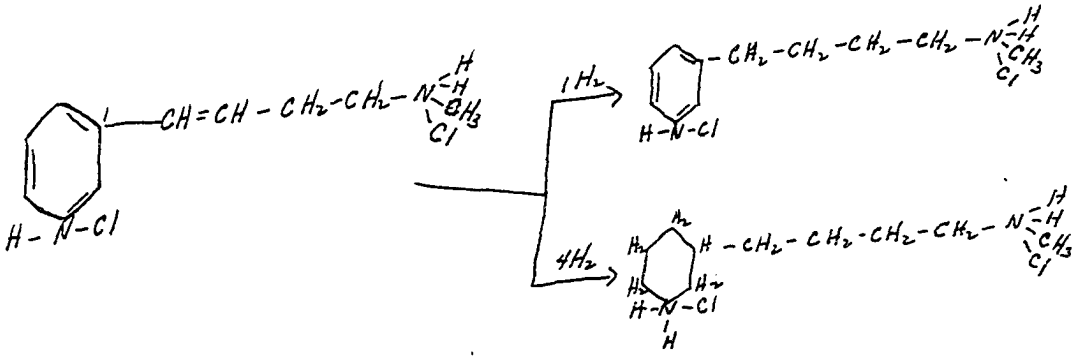
Experimental

For the catalytic reduction of metanicotine, the hydrochloride must be prepared as the free base poisons the catalyst as does nicotine. The hydrochloride was prepared and recrystallized in a similar manner to that employed for nicotine. The recrystallization serves to remove any small amounts of nicotine hydrochloride which might be present. However, nicotine may be removed from the benzoyl metanicotine by washing with water weakly acidified with acetic acid. The acetic acid solution is not acid enough to react with the weakly basic benzoyl metanicotine.

As a standard run 0.1 mole (23.5 gm.) of metanicotine hydrochloride in 150 cc. of 95% ethyl alcohol with 0.2 gm. of platinum-oxide platinum black prepared from C.P. chloroplatinic acid was used. The catalyst was added to the reduction mixture without previous reduction with hydrogen. The time required for reduction to dihydrometanicotine hydrochloride varied from 20 to 30 minutes, the time required for complete

reduction to octohydrometanicotine varied from three to four hours. The dihydrogenated compound is obtained by stopping the reduction when the calculated quantity of hydrogen has been absorbed. The yields in both reductions were practically quantitative.

The reduction may be illustrated structurally:



The reduction to dihydrometanicotine is not particularly sharp. A small amount of octohydrometanicotine may be obtained even though there is still some unreduced metanicotine present. However, the overlapping of the reduction is very slight.

The free bases were obtained from the hydrochlorides, after removal of the catalyst by filtration and evaporating most of the alcohol, by treating with strong sodium hydroxide and extracting with ether. After evaporating the ether the bases may be distilled under atmospheric pressure.

The following table gives the constants for the reduced unreduced products together with a derivative and references.

Table V

Compound	Hydrochloride M.P. °C.	Ref.	Base B.P. °C.	Ref.	Derivative M.P. °C.	Ref.
$C_{10}H_{14}N_2.2HCl$	---		276	(b)	255 (Chloroplatinate)	(b)
$C_{10}H_{16}N_2.2HCl$	---		259	(c)	199 (Chloroplatinate	(c)
$C_{10}H_{22}N_2.2HCl$	202°	(a)	259	(a)	144 (Dibenzene sulfonyl chloride)	(d)

(a) Blau, Ber. 26, 628 (1893).
(b) Pinner, Ber. 27, 1063 (1894).
(c) Loffler and Kober, Ber. 42, 3431 (1909).
(d) Blau, Ber. 26, 1029 (1893).

The picrates were not used for identification of the reduction products as the picrate of both dihydrometanicotine and metanicotine melt at approximately the same place, 162° and 163°C. The picrate of metanicotine may be distinguished from that of dihydrometanicotine if both are recrystallized from water. Metanicotine forms a hydrated picrate which if heated rapidly melts at 114°C. and then resolidifies and melts again at 162°C. Dihydrometanicotine picrate does not form a hydrate and consequently melts at 162° without a previous melting.

TOXICITY TESTS

Introduction

Two methods have been employed in the laboratory testing of contact insecticides when applied in the form of a solution, one method depending upon dipping the insect in solutions of varying concentration, the other depending upon spraying the insect with solutions of varying concentration.

Tattersfield (46) and co-workers used the dipping method extensively in their investigation of the insecticidal properties of various contact insecticides. The procedure which they employed involves the suspension of a definite concentration of the chemical compound in saponin and water. The insects are immersed for ten seconds (a longer time may be used) in 50 cc. of this solution. They are then rapidly transferred to an open vessel containing a piece of filter paper in the bottom to remove excess liquid from the insect. The appropriate food supply of the particular species of insect is placed in the vessel. They tabulate the effects of the insecticide over a period of several days under the following four headings: (1) the killed, (2) the moribund, (3) the slightly affected and (4) the unaffected. A similar method has been employed by Richardson and Smith (32).

(46) Fryer, Stenton, Tattersfield and Roach, Ann. App. Biol. 10, 18 (1923).

A spraying method has also been employed by Tattersfield and Morris (47). This consists of an apparatus designed to spray a known volume of liquid under constant pressure over a constant period of time. After spraying the insects are placed near their appropriate food supply and the same observations recorded as in the dipping method previously referred to.

The spraying method has the advantage over the dipping method in that it can be used for any type of insect while the dipping method cannot be used for sucking insects. With the dipping method, the time of immersion must be constant. Uniform wetting is difficult to obtain.

Experimental

The dipping method was used in a portion of these investigations while the spraying method was used in the remainder. For the spraying tests an apparatus designed by Mr. Roy Melvin, Entomology Department, Iowa State College was used. It consists essentially of a Vilbiss Atomizer No. 16 and a revolving drum enclosed in a framework, the sides of which are pane glass placed in grooves so that they may be moved horizontally to open or close the case. The atomizer is connected to an air pressure line or air pump, the pressure of which may be

(47) Tattersfield and Morris, Bull. Entomol. Res. 14, 223 (1924).

varied. The drum may be revolved at various rates thus giving a means of controlling the volume of spray applied per unit area. This, of course, may also be controlled by the pressure on the atomizer. Clamped on the drum are two wooden strips to which a leaf with the insects feeding upon it may be pinned.

This apparatus eliminates any difficulties which may be encountered in the dipping method due to the variation in time of immersion.

When a constant pressure on the atomizer is used and the drum is rotated at the same rate, the volume of spray striking each individual insect, the size of the individual spray particles, and the "angle of contact" of the spray with the insect are all constant. These factors must be taken into consideration in toxicity tests.

For the determination of the relative toxicities of nicotine and the several related compounds larvae of the Colorado potato beetle (Leptinotarsa decemlineata Say) were used. A number of beetles were collected and caged over potato plants. Eggs were collected every twenty-four hours and kept in separate containers. An average of four and one-half days were required for the eggs to hatch. The larval stage lasts from ten to twelve days. The larvae in all of these tests were used when five days old, this eliminating any biological vari-

ation in individuals due to age. It also allows sufficient time before pupation to make observations three or four days after spraying.

Ten larvae were placed on a potato leaf and immediately started feeding. This leaf was pinned to the drum, the air pressure on the atomizer turned on and the drum started at the same time. The leaf containing the larvae was always placed on the opposite side of the drum from the atomizer so that no spray would hit the larvae before the drum began to rotate. The drum was allowed to make a complete revolution before the pressure on the atomizer was turned off. In all tests the rate of revolution of the drum was kept constant as was the volume of air per unit time passing through the atomizer.

After spraying the larvae were removed from the sprayed leaf and placed on fresh foliage. Mortality counts were made twenty-four and forty-eight hours after spraying. In general these counts showed the same percentage mortality. All tests were made in triplicate at least and most of them five or six times. The average results of these determinations for the different compounds tested are reported in Table VI.

Table VI

(a) Concentration	Nicotine	(b) Nicotine Sulfate	(c) Nicotine Salicylate	Metanicotine	Hexahydronicotine	Octohydronicotine	Piperidine	Pyridine	(d) Pyrrole
	%Kill	%Kill	% Kill	%Kill	%Kill	%Kill	% Kill	% Kill	%Kill
0.1 %	10	6	10						
0.2	40	30	20						
0.3	60	40	35						
0.4	80	54	40						
0.5	100	70	50						
0.6	100	90	60						
0.8	100	100	90	00					
1.0		100	100	00	00				
3.0				00	5.0	00	00		
5.0				15	10.0	00	00		
10.0				40	20.0	10	00	00	00
15.0				95	40.0			00	
20.0						25	10	00	00
Wet control (e)	0								
Dry control (f)	0								

(a) All concentrations refer to the per cent of active constituent rather than the per cent of the compound itself, i.e. 0.1% nicotine sulfate solution contains 0.1 gm. of nicotine as nicotine sulfate per 100 cm. of H_2O.

(b) Pure nicotine neutralized with H_2SO_4 until acid to litmus but slightly basic to methyl orange.

(c) Nicotine salicylate was made by precipitating pure nicotine in ether solution with an ether solution of salicylic acid. Analysis shows 53.34% nicotine corresponding to a monosalicylate. A water solution shows a slight acid reaction to litmus.

(d) Emulsion with 0.1% gelatin.

(e) Wet control sprayed with distilled water.

(f) Not sprayed.

Table VII illustrates a complete set of data for nicotine from which the averages in Table V were compiled. When three determinations or more checked, the results were expressed in round numbers agreeing with the majority of these tests rather than as actual averages. Concentrations are expressed as in Table V.

Table VII

Concentration: Nicotine	Percentage Kill: Test 1	Test 2	Test 3	Test 4	Test 5	Average
0.1	10	10	10	10	00	10
0.2	40	30	44(a)	40	40	40
0.3	60	63(b)	60	60	66(a)	60
0.4	88(a)	70	80	80	77(a)	80
0.5	100	100	100	--	--	100
0.6	100	100	--	--	--	100
0.8	100	--	--	--	--	100

(a) Nine larvae instead of ten.
(b) Eight larvae instead of ten.

Tests on dihydrometanicotine indicate that it is less toxic than metanicotine but more toxic than hexahydronicotine. The data for this compound is limited to two series of tests and is not included in Table VI.

Nicotine tannate is a comparatively insoluble nicotine salt prepared by adding a water solution of nicotine to a saturated water solution of tannic acid. Analysis showed nicotine corresponding to a ditannate. Preliminary tests indicate that is may have some value as a stomach poison. It is also soluble enough to retain toxicity as a contact insecticide. This compound apparently has good sticking properties which recommend it as a spraying agent.

Several tests were made to determine the relative toxicity of nicotine and nicotine sulfate by the dipping method. The method of Tattersfield (46) was used with the exception of the time of immersion, this being increased from ten seconds to one minute. Fifty rice weevils (Calendra oryzae L.) were used in these tests. Mortality counts were made thirty hours after dipping.

The results of these tests are shown in Table VIII.

Table VIII

Toxicity of Nicotine and Nicotine Sulfate

Concentration gm./cc. solution	Percentage Kill	
	Nicotine	Nicotine Sulfate
0.001	6%	8%
0.002	18	9
0.003	30	17
0.004	55	33
0.005	72	50
0.006	89	77
0.007	98	95
0.008	100	100

Fumigation tests were made on the rice weevil (Calendra oryzae L.) using several different concentrations of nicotine vapor, over varying periods of time. The apparatus was similar to that used in determining vapor concentrations of nicotine over a dust. Concentrations were altered by varying the velocity of the air passing over the dust and by using bento-

nite to decrease the volatility. The temperature was maintained at 35°C. and the humidity adjusted to 70 per cent relative humidity. One hundred to one hundred and fifty weevils were used in each test.

After exposing the weevils to the vapor for the desired period of time, they were removed to a clean open container. Mortality counts were made thirty hours after the fumigation.

The data are shown in Table IX.

Table IX

Nicotine Fumigation Tests

Time of Exposure Hours	Percentage Kill		
	Concentration 1.82 mg./10 L.	Concentration 2.48 mg./10 L.	Concentration 2.67 mg./10 L.
0.5		17.0	31.0
1.0	12.0	25.0	54.0
1.5		38.0	
2.0	21.0	73.0	84.0
3.0	39.0	88.0	92.0
4.0	55.0	94.0	96.0
5.0	73.0	99.0	100.0
6.0	90.0	100.0	100.0
7.0	98.0		
8.0	100.0		

Discussion of Results

While both pyrrole and pyridine are relatively non-toxic to insects, hydrogenation of both molecules increases their toxicity. However, the hydrogenated compounds are distinctly less toxic than nicotine.

In these studies it has been shown that hydrogenation of nicotine to hexahydronicotine decreases toxicity almost one hundred-fold. Rupture of the methyl pyrollidine ring without hydrogenation (metanicotine) decreased toxicity but not so markedly as hydrogenation of the pyridine ring. Hydrogenation of the double bond in metanicotine decreased toxicity and finally hydrogenation of both the double bond and pyridine ring in metanicotine lowered the toxicity still further. The order of toxicity as shown in Table VI is nicotine metanicotine dihydrometanicotine hexahydronicotine octohydrometanicotine.

LaForge (33) reports pyridine derivatives of the type of β-pyridylbutyl amine to be of a low order of toxicity as contact insecticides. The α-methyl, α,α'-dimethyl, and α-phenyl pyrrolidines and their N-methyl derivatives (34) were also of a low order of toxicity to insects.

From the foregoing considerations it would appear that the toxicity of nicotine is intimately bound up in the mole-

cule as a whole rather than in any specific part of it. In the literature it has been stated that *d*-nicotine is less toxic to the higher animals than *l*-nicotine (17). Nicotine is peculiar in that the salts of the *l*-nicotine are dextro-rotatory while those of *d*-nicotine are laevo-rotatory. It may be that the toxicity of nicotine is intimately connected with its spafial configuration as well as its constitution. Hexahydronicotine and octohydrometanicotine both contain an asymmetric carbon atom and should be optically active, although neither has been resolved.

The dissociation constants of some of the substituted pyridines are reported in the literature (48). From the toxicities of the few compounds of this nature reported there appears to be a relationship between the ionization constant of the base and its toxicity. There is not sufficient data on either the dissociation constants or the toxicity of these compounds to warrant any conclusions, however. It is interesting to note that the dissociation constant of the pyrrolidine nitrogen in nicotine is reported as 7×10^{-7} (49). There are practically no dissociation constants of organic nitrogen compounds falling between 10^{-6} and 10^{-8} reported in the liter-

(48) Scudder, Conductivities and Ionization Constants of Organic Compounds (D. Van Nostrand Co., 1914).
(49) Kolthoff, Biochem. Z., 162, 289 (1925).

ature; they apparently being relatively unstable. Rupture of the pyrrolidine ring by hydrogenation and also by benzoyl chloride or acetyl chloride indicates the instability of this portion of the nicotine molecule. This may prove to be significant in the physiological action of nicotine.

The relative toxicity of nicotine sulfate and nicotine as contact insecticides indicates that nicotine may gain entrance into the body of the insect in some other way than as the vapor. While nicotine sulfate, being the salt of a weak base and a strong acid, must have a comparatively high hydrolysis constant, the volatility of the nicotine from the sulfate solution, however, is very low compared to that from a free nicotine solution. Most of the work on penetration of nicotine solutions into the body of insects has been with solutions of a dilution of 1:200 or greater. It is possible that after concentration of the nicotine solutions by evaporation the surface tension would be so lowered that it might gain entrance through the tracheae as the liquid rather than the vapor. Since nicotine has such a low vapor pressure at ordinary temperatures this concentration would take place. Tests for nicotine as a residue may be easily obtained upon allowing a 1:200 solution of nicotine to evaporate under atmospheric conditions. It is possible that nicotine sulfate gains entrance in this way. De Ong (14), however, reports

a large difference in the minimum lethal dose of nicotine and nicotine sulfate when administered orally to chickens.

A large variation in the rate of action of nicotine and nicotine sulfate is noted in both the spraying and dipping tests. In the spraying tests the larvae tested become inactive after approximately one minute while with nicotine sulfate five minutes or longer is required before the same condition is reached. The same generalization holds in the dipping tests.

SUMMARY

1. Nicotine has been reduced catalytically with hydrogen and platinum-oxide platinum black to give both hexahydronicotine and octohydrometanicotine.

2. Metanicotine has been reduced catalytically to both dihydrometanicotine and octohydrometanicotine.

3. Hexahydronicotine, octohydrometanicotine, metanicotine and dihydrometanicotine are much less toxic to insects (Leptinotarsa decemlineata) than nicotine.

4. There is a small difference in toxicity between nicotine, nicotine sulfate, and nicotine salicylate but not sufficient to warrant the statement that the toxicity of nicotine as a contact insecticide is due to the vapor entirely.

5. The toxicity of nicotine vapor has been determined for the rice weevil (Calendar oryzae L.).